Chapter 2

Literature Reviews

Fossil fuels are fuels formed by natural processes such as anaerobic decomposition of buried dead organisms and include coal, petroleum and natural gas (Fossil fuel, 2014). Currently, the fossil-based resources, such as natural gas, gasoline and petro-diesel are limited and insufficient for the future world's energy demands. So, there is a need to find out an alternative fuel to fulfill the energy demand of the world. Now, biofuels have increased in popularity because of rising oil prices and the need for energy security.

Biodiesel is one of biofuel. It is produced from oils or fats using transesterification and is a liquid similar in composition to fossil/mineral diesel (Biodiesel, 2014). Feedstock for biodiesel includes animal fats and several types of vegetable oils for example sunflower, palm and Jatropha oil or Physic nut oil. However, shortage of edible oil for human consumption in developing countries does not favor its use for bio-diesel production i.e. sunflower and palm oil. Several years ago, several groups in various sectors are conducting research on *J. curcas* L. or physic nut, a poisonous shrub-like tree that produces seeds considered by many to be a viable source of biofuels feedstock oil because it is non-edible and thus does not compromise the edible oils, which are mainly used for food consumption. Further, Physic nut seed has a high content of oil and the biodiesel produced has similar properties to that of petroleum-based diesel (Koh and Ghazi, 2011). However, it still has some limitations, i.e. difficult harvest and low yield. Nowadays, *in vitro* plant cell culture techniques represent a potential renewable source of valuable medicinals, flavours, essences and colourants that cannot be produced by microbial cells or chemical syntheses (Discomo and Misawa, 1995). Plant cell suspension culture is the one of plant cell culture techniques which has been known to produce plant metabolites such as lipids and might be ability to grow continuously. Therefore, this technique is appropriate for this study and it is potential to be applies on a large scale by using a bioreactor.

The purpose of this review is to provide information about its and current development in the field of plant cell culture research in physic nut.

1. General characteristic of Physic nut

Physic nutor *Jatropha curcas* L.is a species of flowering plant in the spurge family, Euphorbiaceae, that is native to the American tropics, most likely Mexico and Central America. It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas. The specific epithet, "curcas", was first used by Portuguese doctor Garcia de Orta more than 400 years ago and is of uncertain origin. Common names include Barbados Nut, Purging Nut, Physic Nut, or JCL (abbreviation of *J.curcas* L.). It is gaining lot of importance for the production of biodiesel. In addition, it is a tropical plant that can be grown in low to high rainfall areas either in the farms as a commercial crop or on the boundaries as a hedge to protect fields from grazing animals and to prevent erosion (Kumar and Sharma, 2008).

Physic nut, by definition, is a small tree or large shrub, which can be ranch 3-5 meter, but under favorable conditions it can attain a height of eight or ten meters (figure 1A). The plant shows articulated growth, with a morphological discontinuity at each increment. The branches contain latex. Normally, five roots are formed from seedlings, one central and four peripheral. A tap root is not usually formed by vegetatively propagated plants (figure 1B). Leaves with five to seven lobed, hypostomatic and stomata are of paracytic (Rubiaceous) type (figure 1C). The trees are deciduous, shedding the leaves in dry season. Flowering occurs during the wet season and two flowering peaks are often seen, i.e. during summer and autumn. In permanently humid regions, flowering occurs throughout the year. The inflorescence is axillary paniculate polychasial cymes (figure 1D). The plant is monoecious and flowers are unisexual; occasionally hermaphrodite flowers occur. A flower is formed terminally, individually, with female flowers (tricarpellary, syncarpous with trilocular ovary) usually slightly larger and occurs in the hot seasons. In conditions where continuous growth occurs, an unbalance of pistillate or staminate flower production results in a higher number of female flowers. Ten stamens are arranged in two distinct whorls of five each in a single column in the and roecium, and in close proximity to each other. In the gynoecium, the three slender styles are connate to about two-thirds of their length, dilating to massive

bifurcate stigma. The rare hermaphrodite flowers can be self-pollinating. The flowers are pollinated by insects especially honey bees. Each inflorescence yields a bunch of approximately ten or more ovoid fruits (figure 1E). With good rainfall conditions nursery plants may bear fruits after the first rainy season, and directly sown plants after the second rainy season. Three, bivalved cocci is formed after the seeds mature and the fleshy exocarp dries. The seeds mature about 3-4 months after flowering. The seeds are black and the seed weight per 1000 is about 727 g, there are 1375 seeds/kg in the average (figure 1F). Physic nut is a diploid species with $2n = 22$ chromosomes (Kumar and Sharma, 2008).

Physic nut is an excellent hedging plant generally grown in most part of India as live fence for protection of agricultural fields against damage by livestock as unpalatable to cattle and goats. The oil and aqueous extract from oil has potential as an insecticide. For instance it has been used in the control of insect pests of cotton including cotton bollworm and on pests of pulses, potato and corn. All parts of jatropha (seeds, leaves and bark) have been used in traditional medicine and for veterinary purposes for a long time. Some compounds (Curcacycline A) with antitumor activities were reportedly found in this plant. Furthermore, it showed that various solvent extracts of Physic nut have an abortive effect. The oil has a strong purgative action and is also widely used for skin diseases and to soothe pain such as that caused by rheumatism. The latex itself has been found to be strong inhibitors to watermelon mosaic virus. The leaves and latex are used in healing of wounds, refractory ulcers, and septic gums and as a styptic in cuts and bruises. A proteolytic enzyme (curcain) has been reported to have wound healing activity in mice.

The oil from physic nut is regarded as a potential fuel substitute. The types of fuels, which can be obtained directly from the Jatropha plant are; wood, the whole fruit and parts of the fruit which can be burnt separately or in combination (Kumar and Sharma, 2008).

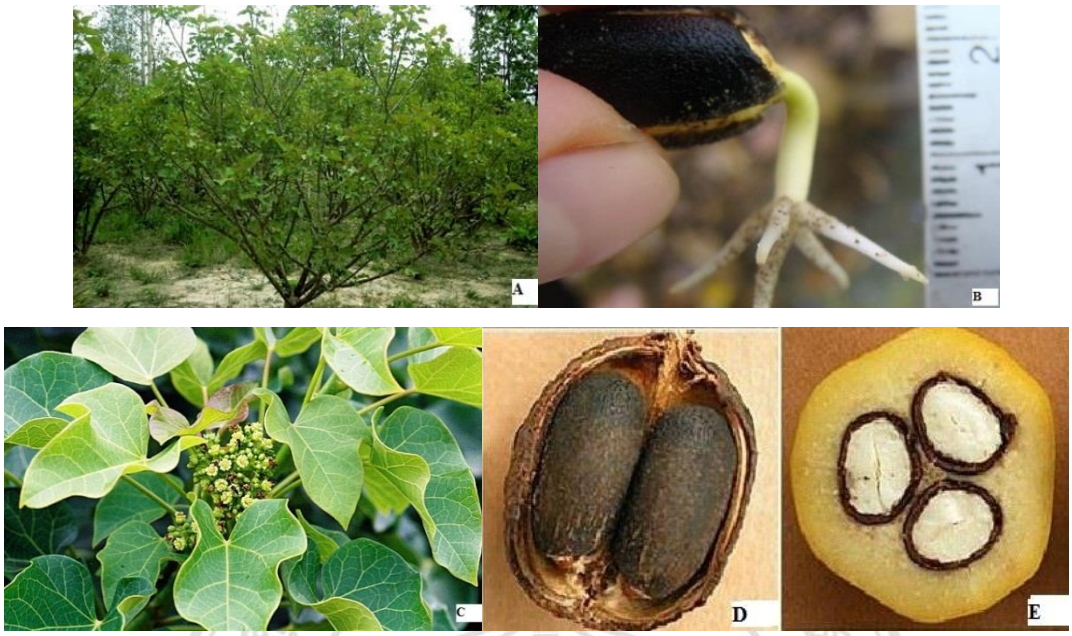

Figure 1 Physic nut

A : plant, B : root, C : leaves and flowers, E : mature fruit, F : fruit and mature seed

Source : A; Chanty, 2014, B; Unknown, 2014, C; Vossen and Mkamilo, 2014, D and E ; Levitan, 2014

2. Seed oil

Seed oil is a vegetable oil that is obtained from the seed (endosperm) of some plant, rather than the fruit (pericarp) (Seed oil, 2014). A vegetable oil is a triglyceride (figure 2) extracted from a plant. The term “vegetable oil” can be narrowly defined as referring only to plant oils that are liquid at room temperature or broadly defined without regard to a substance's state of matter at a given temperature. For this reason, vegetable oils that are solid at room temperature are sometimes called “vegetable fats” (Vegetable oil, 2014). Vegetable oil is an alternative fuel for diesel engines and for heating oil burners. For engines designed to burn diesel fuel, the viscosity of vegetable oil must be lowered to allow for proper atomization of the fuel; otherwise incomplete combustion and carbon build up will ultimately damage the engine (Vegetable oil fuel, 2014).

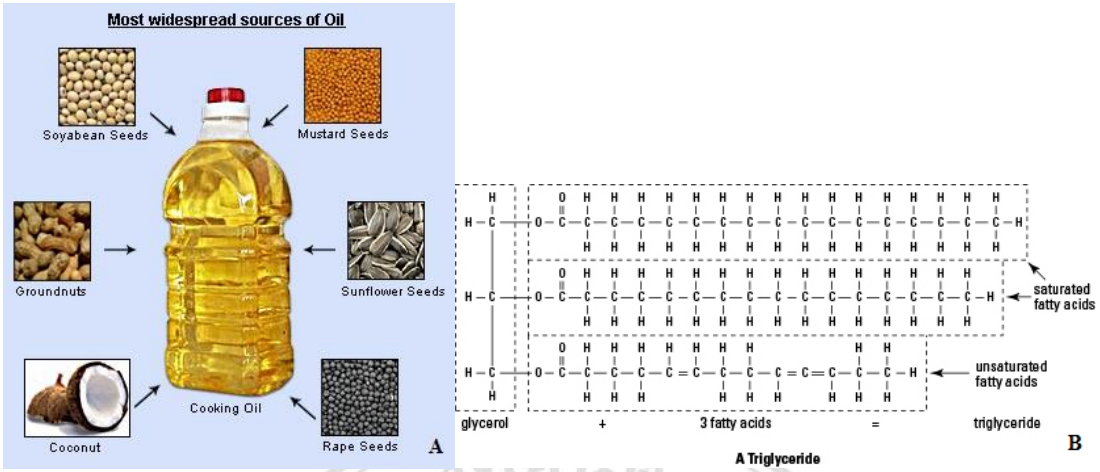

Figure 2 Vegetable oil (A) and Triglyceride (B)

Source : A; Corn Oil Machine, 2014, B; Cliffs Notes, 2014

Chemically, Most vegetable oils are triglycerides (TGs; triglyceride = TG). Chemically, TGs are the triacylglyceryl esters of various fatty acids with glycerol (Knothe *et al*., 2014) (figure 3). The compositions of the lipids of plant tissues are listed in table 1. Triacylglycerols tend to be the most abundant class of storage lipid in tissues that are rich in lipids, such as the commercially important oil seeds. It should be recognized that seeds and tubers do not have a homogeneous lipid distribution, the endosperm, germ, bran and other organelles each having a distinctive composition. In addition, the fatty acid compositions of the seed oils of importance to commerce are listed in table 2 (The AOCS Lipid Library, 2014).

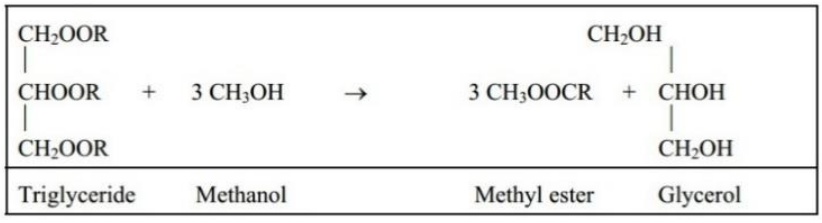

Figure 3 Structure of triglycerides and principle of the transesterification reaction

Source : Knothe *et al*., 2014

Table 1 The lipid class compositions (weight % of the total lipids) of various plant tissues.

Lipid class	Potato tuber	Apple fruit	Soybean seed	Clover leaves	Rye grass	Spinach cloroplasts
monogalactosyldiacylglycerol	6	1	trace	46	39	36
digalactosyldiacylglycerol	16	5	trace	28	29	20
sulfoquinovosyldiacylglycerol	1	1	trace	4	4	5
triglycerol	15	5	88	-	-	-
phosphatidylcholine	26	23	4	7	10	7
phosphatidylethanolamine	13	11	2	5	5	3
phosphatidylinositol	6	6	2	1	2	2
Phosphatidylglycerol	1	1	trace	6	7	7
others	15	42	5	3	4	-

Source : The AOCS Lipid Library, 2014

Table 2 The fatty acid compositions (weight % of the total) of some seed oils.

Fatty acid	**Seed oil**					
	Soybean	**Maize**	**Sunflower**	**Rapseed**[a]	**Olive**	**plam**
16 : 0	11	11	6	3	12	42
18 : 0	4	Trace	3	1	2	4
18 : 1	23	25	12	11	72	38
18 : 2	51	57	73	13	8	9
18 : 3	7	1	1	9	1	-
C_{20}-C_{22}	-	-	-	55	-	-

[a]newers cultivars can contain much less erucic acid

Source : The AOCS Lipid Library, 2014

Some physical properties of the most common fatty acids occurring in vegetable oils and animal fats as well as their methyl esters are listed in Table 3. Besides these fattyacids, numerous other fatty acids occur in vegetable oils and animal fats, but their abundance usually is considerably lower. Table 4 lists the fatty acid composition of some vegetable oils and animal fats that have been studied as sources of biodiesel (Knothe *et al*., 2014). The most common derivatives of TGs (or fatty acids) for fuels are

methyl esters. These are formed by transesterification of the TG with methanol in presence of usually a basic catalyst to give the methyl ester and glycerol (table 5). Other alcohols have been used to generate esters, for example, the ethyl, propyl, and butyl esters (Knothe *et al*., 2014).

Table 3 Selected properties of some common fatty acids and esters.

Trivial (Systematic) name[a] ; Acronym[b]	Mol.wt.	m.p.[c] (°C)	b.p.[c,d] (°C)	Cetane No.	Heat of Combustion[e] (kg-cal/mole)
Caprylic acid	144.22	16.5	239.3		
Lauric acid (Dodecanoic acid); 12 : 0	200.32	44	131		1763.25(25ºC)
Meristic acid (Tetradecanoic acid); 14 : 0	238.38	58	252.5		2073.91(25ºC)
Palmitic acid (Hexadecanoic acid); 16 : 0	256.43	63	350		2384.76(25ºC)
Stearic acid (Octadecanoic acid)	284.48	71	360d		2696.12(25ºC)
Oleic acid (9Z-Octadecanoic acid); 18 : 1	282.47	16	286		2657.4(25ºC)
Linoleic acid (9Z, 12Z-Octadecanoic acid);18 : 2	280.45	-5	229-30		
Linolenic acid (9Z,12Z,15Z-Octadecanoic acid); 18 : 3	278.44	-11	230-2		
Metyl Stearate (Metyloctadecanoate); 18 : 0	298.51	39.1	442-3	86.9 (92.1)[f]	2859

a) Z denotes cis configuration.
b) The numbers denote the number of carbons and double bonds. For example, in oleic acid, 18:1 stands for eighteen carbons and one double bond.
c) Melting points and boiling points given in Ref. 28, pp. C-42 to C-553. Melting points and boiling points of 12:0 - 18:0 and 18:3 esters given in Ref. 181.
d) Superscripts in boiling point column denote pressure (mm Hg) at which the boiling point was determined.
e) See Ref. 27.
f) Cetane number from Ref. 21. Number in parentheses indicates purity (%) of the material used for CN determinations as given by the author. Other CNs given in Ref. 21 not tabulated here (purities in parentheses): ethyl caprate (10:0) 51.2 (99.4); ethyl myristate (14:0) 66.9 (99.3); propyl caprate (10:0) 52.9 (98.0); isopropyl caprate (10:0) 46.6 (97.7); butyl caprylate (8:0) 39.6 (98.7); butyl caprate (10:0) 54.6 (98.6); butyl myristate (14:0) 69.4 (99.0).
g) CN from Ref. 17. CNs (lipid combustion quality numbers) deviating from Ref. 21 as given in Ref. 17: Methyl laurate 54, methyl myristate 72, methyl palmitate 91, methyl stearate 159.

Source : Knothe *et al*., 2014

Table 4 Major fatty acids (in wt.-%) of some oils and fats used or tested as alternative diesel fuels.

Oil or Fat	Fatty acid composition (Wt.-%)							
	12:0	14:0	16:0	18:0	18:1	18:2	18:3	22:1
Coconut	44-51	13-18.5	7.5-10.5	1-3	5-8.2	1.0-2.6		
Olive		1.3	7-18.3	1.4-3.3	55.5-84.5	4-19		
Palm		0.6-2.4	32-46.3	4-6.3	37-53	6-12		
Rapeseed		1.5	1-4.7	1-3.5	13-38	9.5-22	1-10	40-64
Sesame		7.2-9.2	5.8-7.7	35-46	35-48			
Soybean		2.3-11	2.4-6	22-30.8	49-53	2-10.5		
Sunflower		3.5-6.5	1.3-5.6	14-43	44-68.7			
Tallow (beef)		3-6	25-37	14-29	26-50	1-2.5		

Source : Knothe *et al*., 2014

3. Physic nut oil

The wood and fruit of physic nut can be used for numerous purposes including fuel. The seeds of physic nut contain viscous oil, which can be used for manufacture of candles and soap, in cosmetics industry, as a diesel/paraffin substitute or extender. This latter use has important implications for meeting the demand for rural energy services and also exploring practical substitutes for fossil fuels to counter greenhouse gas accumulation in the atmosphere. These characteristics along with its versatility make it of vital importance to developing countries (Kumar and Sharma, 2008).

In physic nut, its produces non-edible oil about 30 to 40% of oil, that accumulate mainly in the endosperm of seeds (Gu *et al*., 2012) (figure 4). Physic nut seed oil chemically consists of triacylglycerol with linear fatty acid chain (unbranched) with/without double bonds. It is considered to be a potential biofuel plant as the fatty acid and lipid profile of physic nut oil is highly suitable for use as biodiesel. Rao *et al*. (2008) revealed that the total oil content of physic nut seeds was 32% with a

composition of 97.6% neutral lipids, 0.95% glycolipids and 1.45% phospholipids. The fatty acid composition of total lipids, neutral lipids, phospholipids and glycolipids was also determined and found to contain oleic acid (18:1) and linoleic acids (18:2) as major fatty acids. The phospholipids fraction was further characterized, quantified and found to contain phosphatidyl choline (PC) 60.5%, phosphatidyl inositol (PI) 24% and phosphatidyl ethanolamine (PE) 15.5%.

Physic nut oil or Jatropha oil is looked up on as one of the most appropriate renewable alternative sources of biodiesel in terms of availability and cost (Rashid *et al*., 2010). Recently novel approach is developed for extraction of oil from seed kernel of Jatropha by using enzyme assisted three-phase partitioning of Jatropha oil are summarized in figure 2 (Kumar and Sharma, 2008).

Table 5 Show the properties of diesel, methanol, Physic nut oil and methyl ester of Physic nut oil

Properties	**Diesel**	**Physic nut oil**	**Metyl ester of Physic nut oil**	**Metanol**
Density (kg m^{-3})	840	918.6	880	790
Colorfic value (k)Kg^{-1})	42,490	39,774	38,450	19,674
Viscosity (cst)	4.59	49.93	5.65	-
Cetane number	45-55	44-45	50	3-5
Flash point (°C)	50	240	170	-
Carbon residue (%)	0.1	64	0.5	0.0

Source : Kumar and Sharma, 2008

4. Oil production in plants

Lipids are an essential constituent of all plant cells. The vegetative cells of plants contain ~ 5 to 10% lipid by dry weight. Lipids are the major form of carbon storage in the seeds of many plant species, constituting up to 60% of the dry weight of such seeds. The most abundant types of lipid in most cells, however, are those that derive from the fatty acid and glycerolipid biosynthetic pathway (Ohlrogge and Browse, 1995). In

seeds, lipids accumulate as triacylglycerols (TAGs), which are formed by an extension of the membrane-lipid biosynthetic pathway common to all plant tissues. Storage lipid is synthesized in two stages in developing seeds, firstly through the production of acyl chains by the plastids, followed by their sequential incorporation into glycerolipids by the acyl-transferases of the endoplasmic reticulum (figure 4) (Hoop *et al*., 2004 refer to Ohlrogge and Browse, 1995). The major fatty acids of plants (and most other organisms) have a chain length of 16 or 18 carbons and contain from one to three cis double bonds. Five fatty acids (18:1, 18:2, 18:3, 16:0, and in some species, 16:3) make up over 90% of the acyl chains of the structural glycerolipidsof almost all plant membranes (figure 4).

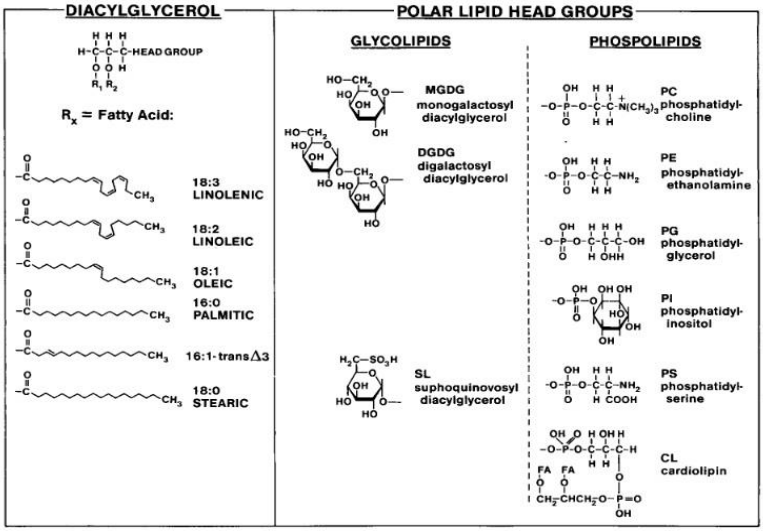

Figure 4 Structures of the Major Fatty Acids and Glycerolipids of Plant Cell Membranes.

The fatty acid and glycerolipid structures are arranged in approximate order of their abundance in plant leaves. Note that the fatty acids are referred to by the number of carbon atoms (before the colon) and the number of double bonds (after the colon).

Source : Ohlrogge and Browse, 1995

The de novo synthesis of fatty acids in plants occurs in the plastids (figure 5) through the activity of fatty acid synthetase. The synthesis of the malonyl-coenzyme A that is required for acyl-chain elongation requires the import of metabolites from the cytosol and their subsequent metabolism. A range of cytosolic metabolites including glucose 6-phosphate, malate, phosphoenol pyruvate and pyruvate support high rates of fatty acid synthesis by isolated plastids, the relative utilisation of which depends upon

the plant species and the organ from which the plastids are isolated. Chloroplasts are able to generate the reducing power and ATP required for fatty acid synthesis by capture of light energy in the reactions of photosynthetic electron transport. Regulation of chloroplast fatty acid synthesis is mediated by the response of acetyl-CoA carboxylase to the redox state of the plastid, which ensures that the carbon metabolism is linked to the energy status. The regulation of fatty acid synthesis in plastids of heterotrophic cells is much less well understood and is of particular interest in the tissues that accumulate large amounts of the storage oil, triacylglycerol. In these heterotrophic cells the plastids import ATP and oxidise imported carbon sources to produce the required reducing power (Stephen, 2002).

The first committed step in fatty acid synthesis is considered to be that catalysed by acetyl-CoA carboxylase (ACCase), which converts acetyl-CoA to malonyl-CoA. Since acetyl-CoA is not imported by plastids it must be generated by metabolism within the plastid. In chloroplasts photosynthesis provides an endogenous source of fixed carbon. Whether this fixed carbon can be utilized for the synthesis of acetyl-CoA depends upon the enzyme complement within the chloroplast. In the case of non-photosynthetic cells/organs the plastid is dependent upon import of metabolites from the cytosol in order to synthesise acetyl-CoA (figure 5). This import process is likely to involve specific transporter proteins on the plastid envelope, as has been reported for non-photosynthetic plastids that carry out starch synthesis. A description of carbon supply to fatty acid synthesis can therefore be broken down into two main sections: (1) the enzymes that synthesize or utilize acetyl-CoA in the plastid; and (2) the uptake and conversion of metabolites into the substrate (s) that these enzymes utilize (Stephen, 2002).

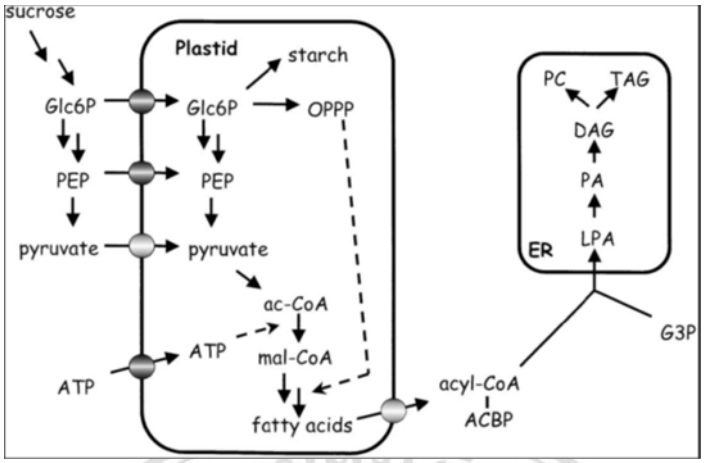

Figure 5 An overview of the current understanding of metabolism associated with fatty acid synthesis and plastidial carbon partitioning in the developing oil seed rape embryo based on metabolic studies.

The scheme presented for Arabidopsis which is based on the detection of ESTs from developing seeds. The compartmental nature of the metabolism from sucrose to lipids involving the cytosol, plastids and the endoplasmic reticulum is illustrated, as is the interaction between the oxidative pentose phosphate pathway and fatty acid synthesis (dashed arrow). Transporters on the plastid envelope are shown as shaded circles. Transporters for which genes have been cloned and their proteins functionally characterized are indicated by darkened shading.
ac – CoA=acetyl-CoA; mal-CoA=malonyl-CoA; OPPP=oxidative pentose phosphate pathway.
Source : Stephen, 2002

5. Plant cell culture in Physic nut.

Plant cell culture is an important tool for basic studies on plant biochemistry and molecular biology, and available methods include regeneration of differentiated cultures (the whole plant and organ cultures; shoots, roots and adventitious roots) or dedifferentiated cultures (e.g., calluses, cell suspensions and protoplasts). Differentiated cultures can be useful for studying tissue-specific biosynthetic pathways, which are not always expressed in cell suspension cultures. In vitro dedifferentiated plant cell suspension cultures are more convenient for the large-scale production of fine chemicals in bioreactors and for the study of cellular and molecular processes as they offer the advantage of a simplified model system for the study of plants because it contains a relatively homogeneous cell population, allowing rapid and uniform access to nutrition, precursors, growth hormones and signal compounds for the cells (Mustafa *et al*., 2011).

The last decade witnessed a blooming interest in the development of in vitro culturing technology for the energy crop Physic nut. Physic nut cell culture studies have been conducted to develop a fast and efficient multiplication system indicate that in vitro plants of Physic nut produce a better yield and yield-related traits than seed propagated plants. Moreover, tissue culture technologies would help in producing the active compounds in vitro with better productivities without cutting down the natural resources. A variety of tissue has been used for the induction of callus and the application of auxin as well as high cytokine in concentrations were effective. Several papers suggest that Physic nut is highly sensitive to auxin with respect to the stimulation of cell division rather than the induction of roots (Attaya *et al*., 2012). In conclusion, in terms of number of callus and hormone reactivity, epicotyls and hypocotyls explants and zygotic embryos were the most suitable explants material. Auxin and cytokinin can independently induce callus with BA and NAA as the most potent hormones. These findings indicated that Physic nut responds to high auxin and high cytokinin primarily by stimulating cell division (Attaya *et al*., 2012).

Some reported on plant cell suspension culture in Jatropha were studied by Soomro and Memon (2007), the establishment of callus and suspension culture in Physic nut Callus cultures were initiated from leaf and hypocotyl explants isolated from 4 days old seedling of Physic nut, on Murashige&Skoog (1962) basal medium supplemented with different growth regulator formulations including 2,4-D, BA, GA3, and coconut milk. Excellent growth of callus was obtained in medium supplemented with 0.5mg/L 2, 4-D alone and with 2% (v/v) coconut milk in hypocotyl explants, Callus produced from hypocotyl explants grew faster during 7 to 30 days of culture then stabilized at a low growth rate. Calli cultured on this medium showed 8 fold increases in fresh weight by the fourth week of incubation. Callus was soft, friable, globular, lush green in color. Hypocotyl explant and 0.5mg/L 2,4-D proved to be most effective in inducing of callus on a large scale in short period of time. The friable green callus was then used for establishment of homogeneous and chlorophyllous suspension culture. Maximum growth of suspension culture was achieved in medium supplemented with 0.5mg/L 2, 4-D, with initial inoculum cell density of 1%. The growth rates of cells were initially slow but as the cultures proceeded, the growth increased significantly and accumulated a great amount of fresh weight (5fold) over a period of 21 days then the

growth of cells was stable for 30 days. The fresh weight was balanced in terms of dry weight which almost corresponded to fresh weight. Total chlorophyll content in cell culture varied between 50.7 to 75.7 µg/g FW with in growth cycle of these cultures. However, Demissie and Lele (2013) reported several researchers have also reported various tissue culture protocols for cultivation of cell suspensions of Jatropha in liquid state for in vitro propagation, germplasm preservation and many transformation systems. Because of it is easy to determine the growth kinetics, the production of metabolites and other physiological activity at a cellular level. Thus, cultivation system in liquid state has remarkable advantages over the conventional solid phase tissue culture and agricultural practice in such regards.

In diploid plants, the endosperm is a triploid (i.e., having 3 sets of chromosomes) tissue as a result of double fertilization, which is a unique process in higher plants (Thomas and Chaturvedi, 2008; Hoshino *et al*., 2011). One of the most important characteristics of triploid plants is seed sterility, and hence, the seed sterility is unfavorable for plants whose seeds are used commercially (Hoshino *et al*., 2011). Polyploid production has been utilized in breeding several crops. Polyploid plants are generally expected to have enlarged organs. In addition, polyploid plants exhibit disease resistance, delayed flowering, or lower fertility in some cases.

Triploid plants are traditionally produced by crossing a diploid plant with an induced tetraploid plant. As compared to conventional methods, endosperm culture provides an easy 1-step protocol for triploid plant production (Thomas and Chaturvedi, 2008). In responding systems, the time needed for triploid plant production is lower than that needed for production using conventional methods. Hence, this method is preferred over conventional techniques. The parenchymatous nature of the endosperm and the absence of vascular tissues make it a unique and excellent experimental system for in vitro culture studies (Thomas and Chaturvedi, 2008; Hoshino*et al*., 2011). The composition of the culture medium is very important for the success of plant regeneration from the endosperm. As a basal medium, the Murashige and Skoog (1962) medium was frequently used. In most cases, callus was induced from cultured endosperm tissues. For callus induction, plant growth regulators (2, 4 dichlorophenoxyacetic acid, 1-naphthaleneacetic acid, or indole-3-butyric acid as auxins; benzylaminopurine or kinetin

as cytokinins) were added to culture media. Endosperm culture is a novel procedure for producing polyploidy plants with endosperm ploidy level. However, researchers need to pay attention to the differences between endosperm culture-derived plants and colchicine treatment-derived polyploidy plants. The significant difference is the genome composition. The endosperm genome is composed of a maternal: paternal genome ratio of 2:1. This affects allelic variations in gene expression, and the differences must occur in endosperm culture-derived plants. Moreover, unique character of endosperm-derived plants is newly produced nuclear-cytoplasmic interactions. The endosperm genome is composed of a maternal: paternal genome ratio of 2:1 and the cytoplasm originates from the maternal side only (Hoshino *et al*., 2011).

Up to date, there is no or limited report for cultivation of Physic nut in a liquid state for any activities and the studies on the oil production from endosperm culture of Physic nut have not been carried out extensively. This dissertation was attempted to study on the oil production from endosperm suspended cell of Physic nut, both in vitro and in the bioreactor. It is hope that the information gained from these studies will provide better understanding in the oil production from endosperm suspended cell of Physic nut, better a formulation of medium and conditions that could be used to induce oil production from endosperm suspended cells in vitro and in the bioreactor. In addition, the results from these studies can be helpful for further plant productivity, bioprocess and biotechnological studies to understand and exploit the potential of Physic nut for various applications in agriculture and industries.